

# Somatic sex determination in *Caenorhabditis elegans* is modulated by SUP-26 repression of *tra-2* translation

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Edited by Judith Kimble, University of Wisconsin, Madison, WI, and approved September 10, 2010 (received for review April 12, 2010)

**Translational repression mediated by RNA-binding proteins or micro RNAs has emerged as a major regulatory mechanism for fine-tuning important biological processes. In *Caenorhabditis elegans*, translational repression of the key sex-determination gene *tra-2* (*tra*, transformer) is controlled by a 28-nucleotide repeat element, the TRA-2/GLI element (TGE), located in its 3' untranslated region (UTR). Mutations that disrupt TGE or the germline-specific TGE-binding factor GLD-1 increase TRA-2 protein expression and inhibit sperm production in hermaphrodites. Here we report the characterization of the *sup-26* gene, which regulates sex determination in the soma and encodes an RNA recognition motif (RRM)-containing protein. We show that SUP-26 regulates the level of the TRA-2 protein through TGE in vivo and binds directly to TGE in vitro through its RRM domain. Interestingly, SUP-26 associates with poly(A)-binding protein 1 (PAB-1) in vivo and may repress *tra-2* expression by inhibiting the translation-stimulating activity of PAB-1. Taken together, our results provide further insight into how mRNA-binding factors repress translation and modulate sexual development in different tissues of *C. elegans*.**

**T**ranslational repression through *cis*-acting elements in mRNAs is an important post-transcriptional regulatory mechanism in numerous biological systems (1). Analysis of the lengths of 5' and 3' untranslated regions (UTRs) shows that the average lengths of 5' UTRs are relatively constant across phyla, whereas the lengths of 3' UTRs increase with organism complexity (200 bp for yeast and 500 bp for humans), suggesting that they may be more highly regulated during animal development (2). Sequence elements or modifications in 3' UTR are known to control the subcellular localization, stability, and translational efficiency of mRNAs. For example, the poly(A) sequence is important not only for the stability of mRNAs, but also for stimulating translation initiation by facilitating interaction of poly(A)-binding (PAB) protein with translation initiation factor eIF4G at the 5' cap and formation of circularized mRNA (1). Moreover, numerous important developmental regulators, such as Bicoid and the cytoplasmic polyadenylation-element binding protein, act by binding 3' UTRs and repressing translation (1). Finally, translational repression by microRNAs (miRNAs) is mediated primarily by formation of nonperfect duplexes between miRNAs and their mRNA targets at 3' UTRs, which induces the formation of the translation repressive complex termed the RNA induced silencing complex (3). Therefore, *cis* elements in the 3' UTR of mRNAs play critical roles in regulating the efficiency of translation.

Sex differentiation in *Caenorhabditis elegans* is determined by the X chromosome:autosome ratio: 1:2 results in XO males and 1:1 results in XX hermaphrodites (4, 5). Hermaphrodites are essentially females that produce sperm before oogenesis and are capable of self-fertilization and mating with other males. Male development is initiated by expression of a male-promoting secreted protein, HER-1 (*hermaphrodization*) (6, 7), which binds and inactivates the hermaphrodite-promoting transmembrane receptor TRA-2 (*TRA*, transformer), which is also important for sperm production (8, 9). TRA-2 interacts with and suppresses the male-promoting activity of

an intracellular protein complex containing FEM-1 (*feminization*), FEM-2, and FEM-3 (10–13). How TRA-2 inhibits the activities of FEM proteins is poorly understood, but it may involve cleavage of the intracellular domain of TRA-2 by the TRA-3 calpain protease and subsequent translocation of the TRA-2 intracellular domain to the nucleus (14–16). The FEM-1/FEM-2/FEM-3 complex promotes male development by inhibiting the activity of the terminal sex-determination factor, TRA-1A, a zinc-finger transcription factor that promotes hermaphrodite development by repressing expression of genes required for sperm production and somatic male development (17, 18).

Translation repression plays an important role in regulating *C. elegans* sex differentiation. For example, the activities of *fem-3* and *tra-2* are regulated by translational repressors acting in specific tissues (19, 20). Several gain-of-function, feminizing mutations in the *tra-2* gene were found to alter the *tra-2* 3' UTR (20, 21). Further studies revealed that two different sequence elements in the *tra-2* 3' UTR regulate TRA-2 translation. First, the TRA-2 retention element retains the *tra-2* message in the nucleus and thus prevents translation (22). Second, the TRA-2/GLI element (TGE) is a conserved 28-nucleotide repeat element found in both *C. elegans tra-2* and *D. melanogaster GLI* 3' UTRs (20, 23). Mutations disrupting TGEs increase *tra-2* poly(A) tail length (23, 24) and TRA-2 protein levels in both the germline and the soma (23, 25), suggesting that TGEs negatively regulate *tra-2* expression. In the germline, repression of *tra-2* translation is mediated by GLD-1 (*germline development defective*), a TGE-binding protein and a member of the STAR family of RNA-binding proteins (25), and by FOG-2 (*feminization of germline*), a GLD-1-interacting and F-box-containing protein (25, 26). FOG-2, GLD-1, and *tra-2* 3' UTR form a ternary complex to repress *tra-2* translation in the germline (26). However, GLD-1 and FOG-2 are expressed only in the germline, and it is unclear how TGEs mediate repression of *tra-2* translation in somatic tissues (26, 27).

Here we report the molecular and biochemical characterization of *sup-26* (*suppressor*). Loss-of-function (*lf*) mutations in *sup-26* are semidominant suppressors of the masculinization defect in *her-1(n695gf)* XX animals and can suppress other masculinization defects in the absence of *her-1*, indicating that *sup-26* likely acts downstream of *her-1* to affect somatic sex determination (28). We find that *sup-26* encodes an RNA recognition motif (RRM) containing protein that is expressed widely in somatic tissues, regulates the level of the *tra-2* protein in the soma through the TGEs in the *tra-2* 3' UTR, and binds directly to TGEs in vitro. Therefore, SUP-26 is a somatic TGE-

Author contributions: J.M. and D.X. designed research; J.M. and J.-T.C. performed research; J.M., J.-T.C., J.-S.Y., and D.X. analyzed data; and J.M. and D.X. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1004513107/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1004513107/-DCSupplemental).

binding factor that promotes male development by repressing *tra-2* translation.

## Results and Discussion

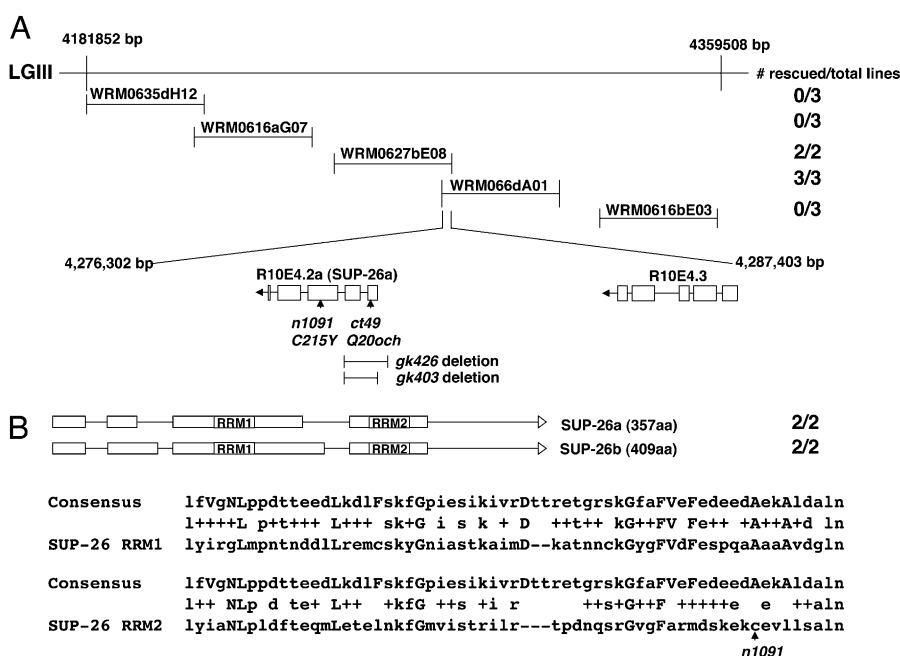
**sup-26 Encodes a Protein with Two RRM Motifs.** *sup-26(n1091)* was isolated as a semidominant suppressor of the masculinized defect of *her-1(n695gf)* XX animals (Table S1) and mapped to a small genetic interval at approximately -3.2 genetic map units on linkage group (LG) III (28). To clone *sup-26*, fosmids covering this genetic interval were injected into *sup-26(n1091)*; *her-1(n695)* animals and tested for restoration of the masculinized Tra phenotype (Fig. 1A). Two overlapping fosmids, WRM0627bE08 and WRM066dA01, each restored the Tra phenotype (Fig. 1A). The overlapping region of the two fosmids contains a single ORF, R10E4.2 (Fig. 1A). A translational GFP fusion that contains a 4-kb genomic fragment, including a 1.1-kb sequence 5' of the R10E4.2 start codon, also restored the Tra phenotype in *sup-26(n1091)*; *her-1(n695)* animals (Table S1; Methods), indicating that R10E4.2 is responsible for the rescuing activity. We determined R10E4.2 DNA sequences from two different *sup-26* mutants, *sup-26(ct49)* and *sup-26(n1091)*, and found a C-to-T transition in *sup-26(ct49)*, which converts codon Q20 to an ochre stop codon, and a G-to-A transition in *sup-26(n1091)*, which converts codon C215 to a tyrosine codon. Two independently isolated deletion mutations, *gk403* (a 424-bp deletion) and *gk426* (a 676-bp deletion), each of which removes the first two exons of R10E4.2 (Fig. 1A), also suppressed the *her-1(n695)* Tra phenotype, confirming that R10E4.2 is *sup-26* (Table S1). Given the molecular nature of the two deletions and *sup-26(ct49)*, they are likely strong *lf* or null mutations. However, *sup-26* mutant males or hermaphrodites alone display no obvious defect in sex determination (Tables S1 and S2). Therefore, *sup-26* appears to be a modulator of the sex-determination pathway, fine-tuning the pathway to ensure appropriate sexual development.

We performed reverse transcription PCR amplification (RT-PCR) with primers corresponding to the predicted 5' and 3' ends of the *sup-26* coding sequence (<http://www.wormbase.org/>) and identified two distinct transcripts, *sup-26a* and *sup-26b*, which encode 357 and 409 amino acid products, respectively (Fig. 1B). The predicted products of both transcripts contain two RRM

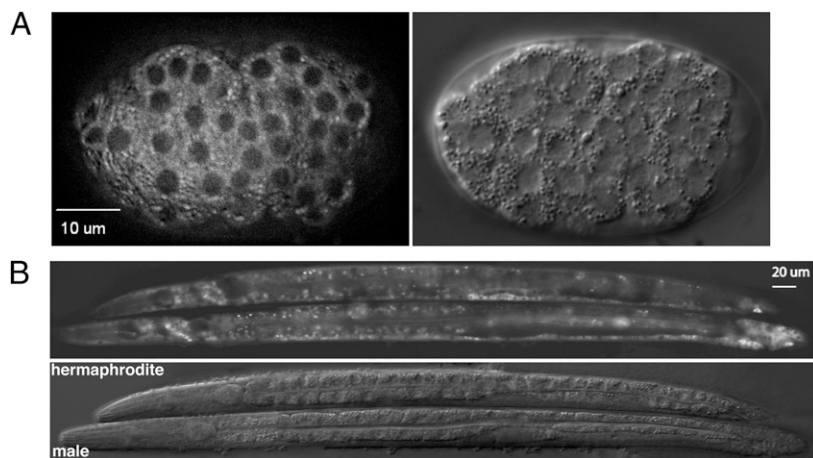
that share 77% and 74% sequence similarity to the consensus RRM sequence, respectively (Fig. 1B), suggesting that SUP-26 may bind RNA. When expressed under the control of the *sup-26* promoter, each of the transcripts masculinized *sup-26(n1091); her-1(n695)* animals (Fig. 1B), indicating that both *sup-26* isoforms are functional.

**SUP-26 Is Broadly Expressed in Somatic Cells and Localizes to the Cytoplasm.** To determine where SUP-26 might function, we examined the expression pattern of the  $P_{sup-26}sup-26::gfp$  translational fusion, which fully rescued the *sup-26(n1091)* phenotype (Table S1). We found that SUP-26::GFP was expressed in most if not all, somatic cells, starting from the early gastrula through adulthood. SUP-26::GFP localized to the cytoplasm and was largely excluded from the nucleus (Fig. 2). There was no apparent difference in SUP-26::GFP expression patterns between male and hermaphrodite L4 larvae or adults (Fig. 2B). Based on data from the Nematode Expression Pattern DataBase (<http://nematode.lab.nig.ac.jp>), in situ hybridization experiments using either *sup-26a* or *sup-26b* cDNA as probes reveal that the *sup-26* messages are absent from early meiotic-stage germ cells but are present in oocytes.

**TRA-2 Protein Expression Is Increased by *sup-26* Loss-of-Function Mutations.** Previous genetic analysis indicates that *sup-26* may regulate sexual development through *tra-2* (28). We thus examined whether *sup-26* mutations affect *tra-2* gene expression. We generated a 15-kb transgene that contains the entire *tra-2* operon (*ppp-1* and *tra-2*), including an 816-bp promoter upstream of *ppp-1*, the first gene of the operon, the coding region of *ppp-1*, the *tra-2*-coding region fused at its carboxyl terminus with GFP or 3xFLAG epitope, and an 848-bp *tra-2* 3' UTR. Stable integration lines were generated from these transgenes: *smIs380* (*P<sub>tra-2</sub>-tra-2::gfp*) and *smIs350* (*P<sub>tra-2</sub>-tra-2::3xflag*) (*Methods*). Both integrated lines fully rescued the *tra-2(lf)* defects (Fig. S1), suggesting that the TRA-2 fusion proteins are functional. Despite being a predicted transmembrane receptor (Fig. 3A) (8), TRA-2::GFP was observed exclusively in the nucleus as previously described (Fig. 3B) (15). Interestingly, TRA-2::GFP was expressed at higher levels in *sup-26(gk426)* animals than in wild-type animals (Fig. 3B). For example, only several cells in the head of wild-type



**Fig. 1.** Cloning of *sup-26*. (A) Fosmids used in *sup-26(n1091)* rescue experiments and their relative base-pair positions on LGIII are shown. Transgenic *sup-26(n1091); her-1(n695)* animals carrying the indicated fosmid DNA as extrachromosomal arrays were generated and scored for restoration of the masculinized (Tra) phenotype as described in *Methods*. The number of rescued lines vs. total lines generated are indicated at the right. ORFs in the overlapping region of two rescuing fosmids (WRM0627bE08 and WRM066dA01) are indicated, with boxes representing exons and lines representing intronic sequences. The positions of *ct40* and *n1091* mutations are indicated by arrows. Two deletion alleles (*gk403* and *gk426*) and the *sup-26* regions removed by these mutations are represented below the *sup-26* ORF. (B) A schematic of *sup-26* transcripts and alignment of the consensus RRM (accession no. PF00076) with the two SUP-26 RRM. Expression of these two transcripts under the control of the *sup-26* promoter rescued the *sup-26(n1091)* phenotype. Uppercase letters indicate the most conserved residues of RRM. The middle rows show residues that are identical (letters) or conservative changes (+). The RRM domains are identical in SUP-26a and SUP-26b. The residue affected by *n1091* is indicated by an arrow.



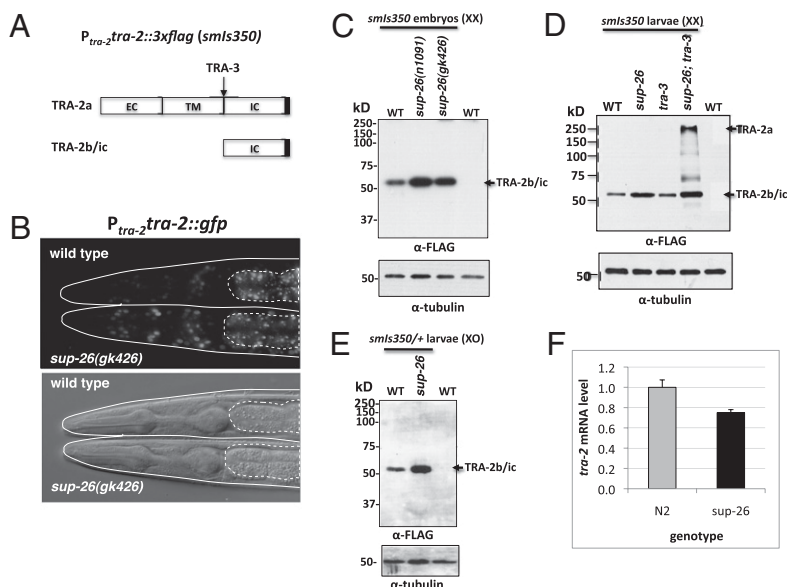
**Fig. 2.** SUP-26 expression patterns in *C. elegans* embryos and larvae. (A) SUP-26::GFP observed in an early gastrula embryo carrying an integrated array containing  $P_{sup-26}sup-26::gfp$  (Left) and the corresponding DIC image of the embryo (Right). (B) Expression of SUP-26::GFP in L4 stage hermaphrodite (Upper) and male (Lower) larvae, respectively. The corresponding DIC image is shown below.

animals displayed TRA-2::GFP, whereas many cells in *sup-26(gk426)* animals expressed TRA-2::GFP. In Western blot analysis of *smIs350* hermaphrodites with different genetic backgrounds, we observed significantly increased levels of an  $\approx 50$ -kDa TRA-2::3xFLAG polypeptide in *sup-26* mutant embryos and L4 larvae compared with those in wild-type embryos and L4 larvae (Fig. 3C and D). This TRA-2::3xFLAG polypeptide is similar in size to the TRA-2 product (TRA-2ic) generated by TRA-3 protease cleavage at the intracellular domain of TRA-2a (14) and to the predicted size of the TRA-2b isoform. It is also consistent with the size of the TRA-2 protein detected in immunoblot analysis using an antibody raised against the TRA-2 intracellular domain (15, 16). In *sup-26(gk426); tra-3(e1107) smIs350* animals, we observed one additional high-molecular-weight form of TRA-2::3xFLAG consistent in size with full-length TRA-2 (Fig. 3D), indicating that TRA-2a is indeed cleaved by TRA-3 in *C. elegans*. In *sup-26(gk426); smIs350/+* males, we observed a similar increase in the abundance of the 50-kDa TRA-2::3xFLAG polypeptide when compared with

wild-type *smIs350/+* males (Fig. 3E), which were mildly feminized due to TRA-2 overexpression from the *smIs350* transgene (Table S2). The feminization phenotype of *sup-26(gk426); smIs350/+* males was stronger than that of *smIs350/+* males, which is consistent with more increased TRA-2 expression in *sup-26(gk426); smIs350/+* males. These results suggest that in both males and hermaphrodites SUP-26 represses *tra-2* protein expression. Moreover, *sup-26* can inhibit translation from both *tra-2* transcripts, which are transcribed from different promoters but share the same 3' UTR (Fig. S1) (8). Real-time quantitative RT-PCR analysis revealed a slight decrease in *tra-2* transcripts in *sup-26(gk426)* mixed-stage animals when compared with wild-type animals, indicating that *sup-26* does not inhibit *tra-2* transcription or reduce *tra-2* mRNA stability (Fig. 3F). Therefore, our results are consistent with the model that *sup-26* regulates *tra-2* expression by inhibiting *tra-2* translation.

**SUP-26 Regulates *tra-2* Expression Through the TGE Elements.** It was previously shown that translation of *tra-2* in the germline is

**Fig. 3.** Analysis of TRA-2 protein expression. (A) A schematic of *tra-2* translation products (TRA-2a and TRA-2b) and the product (TRA-2ic) derived from processing of TRA-2a by the TRA-3 calpain protease (16). The predicted extracellular (EC), transmembrane (TM), and intracellular (IC) domains are indicated. The 3xFLAG is indicated by a solid box. (B) TRA-2::GFP expression in L4 stage wild-type (Upper) and *sup-26(gk426)* (Lower) hermaphrodites carrying an integrated array containing  $P_{tra-2}tra-2::gfp$ . Regions of intestinal auto-fluorescence are bounded by dashed lines. TRA-2::GFP was seen in nuclei of the head region. (Lower) Corresponding DIC image of the Upper panel. (C–E) Immunoblotting analysis of TRA-2 expression from an integrated transgene (*smIs350; P<sub>tra-2</sub>tra-2::3xflag*) in different genetic backgrounds. (C) A total of 250 embryos of the indicated genotype carrying *smIs350* or 250 nontransgenic wild-type (WT) embryos were solubilized with SDS sampling buffer, resolved on 10% SDS/PAGE, and then analyzed by immunoblotting using an anti-FLAG antibody or an anti- $\beta$ -tubulin antibody (as a loading control). (D) Twenty-five L4 larvae of the indicated genotype carrying *smIs350* or a nontransgenic wild-type control were analyzed by immunoblotting as described above. The alleles used were *sup-26(gk426)* and *tra-3(e1107)*. To resolve the high-molecular-weight TRA-2a transmembrane isoform that is prone to aggregate when boiled, the samples were sonicated in the SDS sampling buffer in a water bath and heated at 65 °C for 30 min before being resolved by a 8% SDS/PAGE. (E) Seventeen male L4 larvae of the indicated genotype, which were heterozygous for *smIs350*, were analyzed by 10% SDS/PAGE and immunoblotting as described above. *smIs350* homozygous XO males are slightly feminized. Therefore, *smIs350/+* XO males were used. (F) Abundance of the *tra-2* transcripts in wild-type and *sup-26(gk426)* animals. Quantitative RT-PCR was performed on RNA samples from mixed-stage wild-type (N2) and *sup-26(gk426)* animals. *rpl-26* was used as an internal reference. Mean value of *tra-2* mRNAs is expressed as a ratio over *rpl-26*. Error bars are SDs.

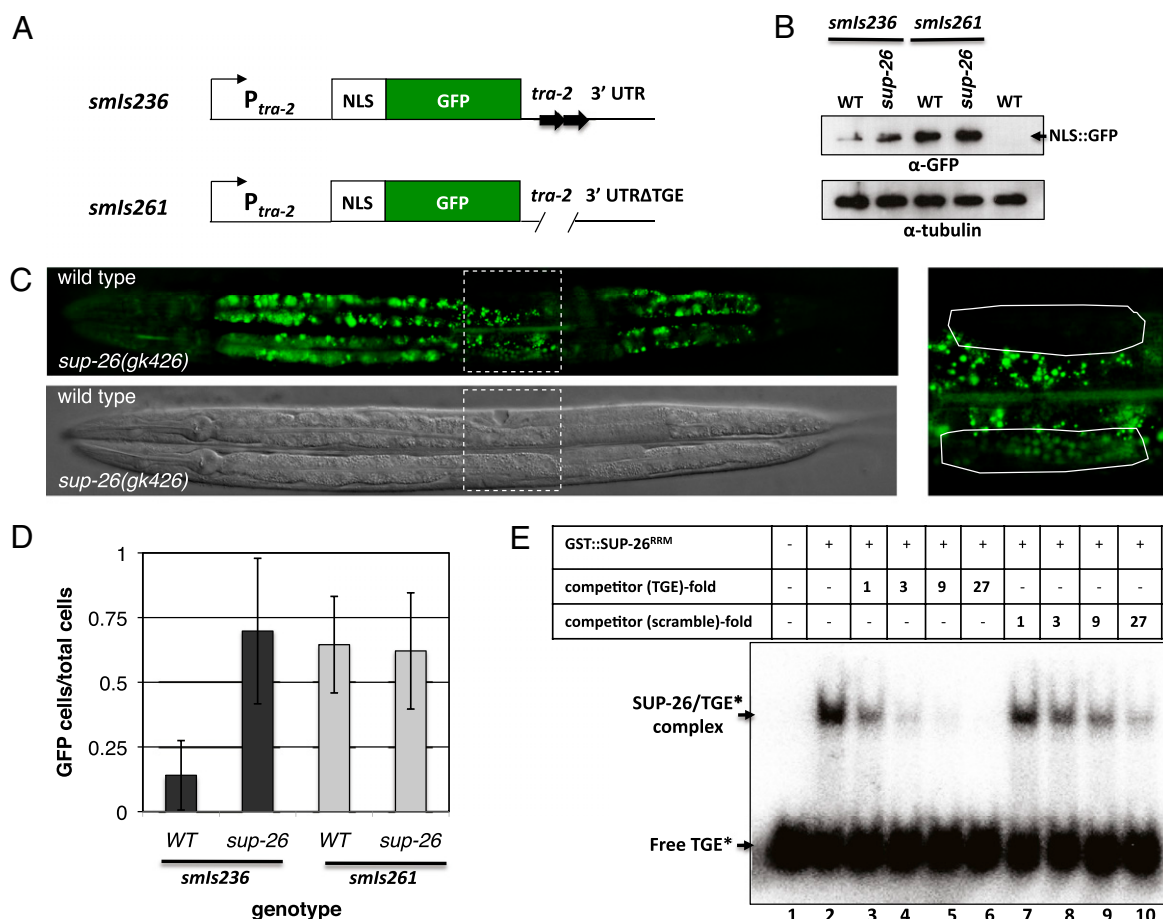




repressed by elements in its 3' UTR (20). We thus tested whether *sup-26* acts through the *tra-2* 3' UTR. We generated GFP reporters that lack the TRA-2 coding sequence but contain the 816-bp *tra-2* promoter, the coding region for nucleus-localized GFP (NLS::GFP), and an 848-bp *tra-2* 3' UTR (Fig. 4A; *Methods*). An integrated transgene, *smIs236* [*P<sub>tra-2</sub>*-NLS::GFP::3'UTR<sub>*tra-2*</sub>], had stronger GFP expression in *sup-26(gk426)* animals than in wild-type animals on the basis of the immunoblotting analysis (Fig. 4B) and the analysis of GFP fluorescence intensity (Fig. 4C). Increased NLS::GFP expression in *sup-26(gk426)* animals was apparent in most tissues and was particularly obvious in the uterus. For example, in *smIs236* animals, an average of 17% uterine cells had visible NLS::GFP expression, compared with an average of 70% in *sup-26(gk426)*; *smIs236* animals (Fig. 4D). In contrast, a similar integrated transgene lacking both 28-bp TGEs, *smIs261* [*P<sub>tra-2</sub>*-NLS::GFP::3'UTR( $\Delta$ TGE)<sub>*tra-2*</sub>], produced similar levels of NLS::GFP expression in *sup-26(gk426)* and wild-type animals (Fig. 4B and D), suggesting that SUP-26 likely inhibits *tra-2* translation through TGEs.

We then tested whether SUP-26 binds directly to the *tra-2* 3' UTR in vitro. We found that a purified SUP-26 GST fusion (GST::SUP-26<sup>RRM</sup>), which contains the SUP-26a RRM domain (amino acids 81–259), formed a complex with a <sup>32</sup>P-labeled TGE RNA oligonucleotide, displaying retarded mobility in a gel shift assay (Fig. 4E, lanes 1 and 2). Unlabeled TGE oligonucleotide competed effectively for binding to GST::SUP-26<sup>RRM</sup> in a concentration-dependent manner, blocking the complex formation (Fig. 4E, lanes 3–6). In contrast, an RNA oligonucleotide with the identical nucleotide composition but a scrambled sequence was much less effective in doing so, showing an approximately ninefold lower binding affinity (Fig. 4E, lanes 7–10). These results suggest that SUP-26 binds specifically to the 3' UTR of the *tra-2* mRNA through the 28-nt TGEs.

**Polyadenylate-Binding Protein Associates with SUP-26 in Vivo.** To identify factors that may act with SUP-26 to regulate *tra-2* translation, we immunoprecipitated SUP-26::GFP from extracts



**Fig. 4.** SUP-26 represses *tra-2* translation by binding to the TGE elements in *tra-2* 3' UTR. (A) A schematic showing two *tra-2* transcriptional fusions used to examine the role of TGEs in regulating *tra-2* expression. A GFP with four copies of the SV40 nucleus localization signal (NLS) is under the control of the *tra-2* promoter and 3' UTR with or without the two TGEs (*Methods*). (B–D) Expression levels of NLS::GFP in wild-type and *sup-26(gk426)* hermaphrodite larvae carrying two different integrated arrays, *smls236* [*P<sub>tra-2</sub>*::NLS::GFP::3'UTR<sub>*tra-2*</sub>] and *smls261* [*P<sub>tra-2</sub>*::NLS::GFP::3'UTR( $\Delta$ TGE)<sub>*tra-2*</sub>], which lacks two TGEs. (B) One hundred larvae of the indicated genotype were analyzed by 12% SDS/PAGE and then by immunoblotting using an anti-GFP antibody or an anti- $\alpha$ -tubulin antibody as described in Fig. 3C. (C) GFP and DIC images of representative L4 stage wild-type (*Upper*) and *sup-26(gk426)* (*Lower*) hermaphrodites carrying *smls236*. The region indicated by the dashed box is enlarged on the right, and the uterine cells bounded by the dashed box were scored for NLS::GFP expression. (D) Percentages of uterine cells that expressed NLS::GFP in wild-type or *sup-26(gk426)* hermaphrodites carrying *smls236* or *smls261*. Images of 15 animals from each strain were captured and scored blind of the genotype for uterine cells with NLS::GFP, which are expressed as a ratio over the total number of uterine cells scored. Error bars are SDs. (E) SUP-26 binds specifically to the TGE element. A <sup>32</sup>P-labeled 28-nt TGE RNA oligonucleotide (1.8 pmol) was incubated with or without 2.1 pmol of purified GST::SUP-26<sup>RRM</sup> (lanes 1 and 2) in the presence or absence of increasing concentrations of unlabeled TGE RNA oligonucleotide (lanes 3–6) or an RNA oligonucleotide with a scrambled TGE sequence (lanes 7–10), whose concentrations are presented as folds of the <sup>32</sup>P-labeled TGE. The reactions were resolved by 5% nondenaturing polyacrylamide gel (*Methods*).

of *P<sub>sup-26</sub>sup-26::gfp* transgenic animals (*Methods*). SDS polyacrylamide gel resolution of proteins coprecipitated with SUP-26::GFP revealed the presence of two major protein bands that were not observed in the mock immunoprecipitation (IP) sample (Fig. 5A). MALDI-TOF mass spectroscopy analysis determined that the lower band (Fig. 5A, band 2) corresponds to SUP-26::GFP and the upper band (Fig. 5A, band 1) corresponds to the poly(A)-binding protein PAB-1 (Table S3), which was confirmed by liquid chromatography-tandem mass spectrometry (LC MS/MS) analysis using LTQ Orbitrap (Fig. S2). To examine whether SUP-26 and PAB-1 directly interact, we performed a GST fusion protein pulldown assay. GST::SUP-26 and GST::SUP-26<sup>C</sup> [which contains the carboxyl terminal domain of SUP-26a (amino acids 260–357), but not GST], GST-SUP-26<sup>RRM</sup>, or GST::SUP-26<sup>N</sup> [which contains the amino terminal domain of SUP-26a (amino acids 1–80)] specifically pulled down His<sub>6</sub>::PAB-1::FLAG in the presence of RNase A (Fig. 5B). These results suggest that SUP-26 and PAB-1 can directly interact in vitro independently of RNA through the carboxyl terminal domain of SUP-26. Finally, we tested whether *sup-26* may affect the length of the poly(A) tail of *tra-2* mRNA using a PCR-based assay (Fig. S3) (23, 24). We were able to detect relatively short poly(A) tails on *tra-2* mRNAs, similar to what has been reported previously (23, 24), but their lengths were not affected by mutations in *sup-26* (Fig. S3B).

In summary, we have identified an RRM-containing protein, SUP-26, that is ubiquitously expressed in *C. elegans* somatic cells, binds specifically to TGEs in the 3' UTR of *tra-2* mRNA, and modulates somatic sex determination by repressing *tra-2* translation. Interestingly, GLD-1, a germ-cell-specific RNA-binding protein that shares no sequence similarity with SUP-26, also binds TGEs in the *tra-2* 3' UTR to repress its translation in the germline and to promote spermatogenesis (25, 26). It appears that GLD-1 and SUP-26 use the same 3' UTR *cis*-element (TGEs) but different cofactors or mechanisms to repress *tra-2* translation. In the germline, FOG-2, a unique F-box protein and a germ-cell-specific factor, is proposed to act as a bridge to bring GLD-1-bound *tra-2* mRNA into a translational repression complex (26). We find that PAB-1, a poly(A)-binding protein, associates with SUP-26 in vivo and interacts directly with SUP-26 in vitro independently of RNA. The PABPs have been shown to interact with translation initiation factors such as eIF4G to form a circular mRNA structure that facilitates active translation (1, 29). Their binding to the poly(A) sequences could also prevent deadenylation and thus stabilize mRNAs (1, 29), although loss of *sup-26* does not appear to affect the lengths of *tra-2* poly(A)

tails. It seems more likely that the association of SUP-26 with PAB-1 at the *tra-2* 3' UTR interferes with PAB-1's function in stimulating *tra-2* translation. If so, this would represent a different TGE-mediated translational repression mechanism from the one used in the germline and perhaps is similar to that used by inhibitory PABP-interacting proteins, which inhibit translation by antagonizing the translation-stimulating activity of PABPs in mammalian cells (30).

## Methods

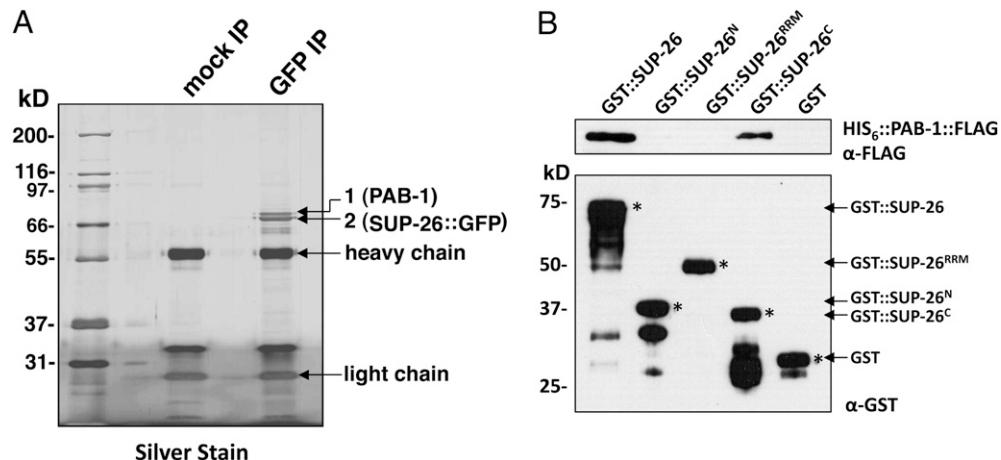
**Strains.** Strains were maintained using standard procedures. Transgenic strains were generated by microinjection (31). Integration of extrachromosomal transgene arrays was performed by the  $\gamma$ -irradiation method (32). Mutations and integrated arrays used in this study were as follows: LGIII—*sup-26(gk426, gk403, n1091, ct49)*; LGIV—*tra-3(e1107)*, *smls350* (*P<sub>tra-2</sub>tra-2::3xflag*), *smls261* [*P<sub>tra-2</sub>NLS::GFP::3'UTR(ΔTGE)<sub>tra-2</sub>*]; LGV—*unc-76(e911)*, *her-1(n695)*; and LGX—*smls236* (*P<sub>tra-2</sub>NLS::GFP::3'UTR<sub>tra-2</sub>*) and *smls259* (*P<sub>sup-26</sub>sup-26::gfp*). The chromosomal location of *smls380* (*P<sub>tra-2</sub>tra-2::gfp*) has not been determined.

**Molecular Biology and Transgenic Animals.** Sequences of all primers used in this study are listed in Table S4. Fosmids were injected into *sup-26(n1091)*; *her-1(n695)* animals at 10 ng/μL using pRF4 as a co-injection marker (50 ng/μL). *P<sub>sup-26</sub>sup-26::gfp* was constructed by PCR amplification of the 4-kb *sup-26* genomic fragment using the primers SUP-26pro and SUP-26cas and by subcloning the PCR fragment into a modified pPD117.01 vector using the standard Gateway cloning technique. *P<sub>sup-26</sub>sup-26::gfp* was injected at 5 ng/μL with pRF4 (50 ng/μL).

The *sup-26* cDNAs were amplified from a cDNA library prepared from mixed-stage wild-type animals using primers complementary to the predicted 5' and 3' ends of the *sup-26*-coding sequence. The amplified cDNA fragments were cloned into the Gateway vector pDONR221. Of 20 cDNA clones analyzed by restriction enzyme digestion and DNA sequencing, 17 were *sup-26a* and 3 were *sup-26b*.

The *P<sub>tra-2</sub>NLS::GFP::3'UTR<sub>tra-2</sub>* reporter was generated by inserting an 816-bp *tra-2* operon promoter fragment (XbaI-XmaI) and an 848-bp *tra-2* 3' UTR fragment (EcoRI/SpeI) into pPD122.56. *P<sub>tra-2</sub>NLS::GFP::3'UTR(ΔTGE)<sub>tra-2</sub>*, which lacks two TGEs, was generated by site-directed mutagenesis. These plasmids were injected individually into *unc-76(e911)* animals at 50 ng/μL with p76-16b (an *unc-76* rescuing plasmid) at 25 ng/μL. To generate *P<sub>tra-2</sub>tra-2::3xflag*, a 12,850-bp genomic fragment containing an 816-bp promoter upstream of *ppp-1*, the coding region of *ppp-1*, and the *tra-2*-coding region were fused to three tandem copies of the FLAG tag (DYKDDHGDYKDDHDI-DYKDDDDK). The 848-bp *tra-2* 3' UTR was then fused to the 3' end of the 3xFLAG tag. *P<sub>tra-2</sub>tra-2::gfp* was made by replacing the 3xFLAG epitope sequence of *P<sub>tra-2</sub>tra-2::3xflag* with a KpnI-EcoRI *gfp* fragment from pPD95.75. *P<sub>tra-2</sub>tra-2::3xflag* or *P<sub>tra-2</sub>tra-2::gfp* was injected into *unc-76(e911)* animals at 25 ng/μL with p76-16b (50 ng/μL).

**Fig. 5.** PAB-1 associates with SUP-26 both in vivo and in vitro. (A) Lysates from *C. elegans* animals expressing SUP-26::GFP (*smls259*) were prepared as described in *Methods*, incubated with a mouse anti-GFP monoclonal antibody (GFP IP) or no antibody (mock IP), precipitated using Protein G Sepharose beads, resolved by 12% SDS/PAGE, and subjected to silver staining. Two major bands not observed in mock IP were excised from the gel, subjected to trypsin digestion, and analyzed by MALDI-TOF mass spectroscopy and LC-MS/MS. The upper band corresponds to PAB-1 and the lower band is SUP-26::GFP. (B) PAB-1 associates with SUP-26 in vitro through the carboxyl-terminal domain of SUP-26 in the presence of RNases. A total of 200 ng of purified GST, GST-SUP-26, GST-SUP-26<sup>N</sup>, GST-SUP-26<sup>RRM</sup>, and GST-SUP-26<sup>C</sup> were incubated with glutathione Sepharose beads and 100 ng of purified His<sub>6</sub>::PAB-1::FLAG. The bead-bound proteins were resolved by 12% SDS/PAGE and analyzed by immunoblotting with anti-GST and anti-FLAG antibodies, respectively. Asterisks indicate the corresponding GST fusion proteins.



**Protein Purification and Gel Mobility Shift Assay.** Gel shift assays were performed as described previously (33). Briefly, GST::SUP-26<sup>RRM</sup> was purified from the BL21(DE3) *Escherichia coli* strain using glutathione Sepharose beads (GE Healthcare). An RNA oligonucleotide corresponding to the 28-nt TGE element was synthesized (Integrated DNA Technologies) and end-labeled with <sup>32</sup>P using polynucleotide kinase (New England Biolabs). For the binding reaction, GST::SUP-26<sup>RRM</sup> was incubated at 25 °C with <sup>32</sup>P-labeled RNA in a binding buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 100 mM KCl, 0.1 mM DTT, 5% glycerol, and 0.1 mg/mL BSA in the presence or absence of unlabeled RNA oligonucleotide competitors. After a 20-min incubation, the samples were resolved on a 5% nondenaturing polyacrylamide gel at 4 °C. The gel was then dried and exposed to a PhosphorImaging screen (Perkin-Elmer).

**Microscopy Imaging.** Fluorescence and differential interference contrast (DIC) images were collected at 0.5-μm intervals with an Axioplan 2 microscope (Zeiss) and a cooled CCD camera (PCO SensiCam). Fluorescence images were subjected to deconvolution analysis using the Slidebook 5.0 software program (Intelligent Imaging Innovations).

**Mass Spectroscopy Analysis.** Mixed-stage animals were harvested from nematode growth media (NGM) agar plates and lysed by sonication (3 × 10 s) in a buffer containing 250 mM NaCl, 100 mM Tris-HCl (pH 7.4), 1 mM EDTA, 5 mM DTT, 0.1% Triton X-100, 1% PMSF, and Roche Complete Protease Inhibitor Mixture. Lysates were clarified by centrifugation at 14,000 × g for 30 min, precleared with Protein G beads (GE Healthcare), and then incubated with an anti-GFP antibody and Protein G beads for 2 h at 4 °C with gentle rocking. After four extensive washes with the same buffer, the precipitated samples were resolved in 12% SDS/PAGE and silver-stained. In-gel tryptic digestion of silver-stained proteins and mass spectrometric analysis were carried out as described (SI Methods) (34).

**GST Fusion Protein Pulldown Assay.** GST-SUP-26 fusion proteins were expressed and purified as described above. His<sub>6</sub>::PAB-1::FLAG expressed in BL21(DE3) was first purified using TALON Metal Affinity Resin (Clontech) and eluted from the resin with 200 mM imidazole. It was further affinity-purified using the anti-FLAG (M2) agarose beads (Sigma-Aldrich) and eluted with 100 μg/mL of the FLAG peptide. His<sub>6</sub>::PAB-1::FLAG was incubated with GST fusion proteins immobilized on glutathione Sepharose beads in the PBS buffer supplemented with 10% glycerol, 1 mM DTT, 0.01% Nonidet P-40, 0.5 mM EDTA, and 125 μg/mL RNase A at 4 °C for 12 h with gentle rotating. The Sepharose beads were washed four times with the binding buffer. The bound proteins were resolved by 12% SDS/PAGE and detected by immunoblotting.

**Poly(A) Tail Length Assay.** The poly(A) tail length assay was carried out on *tra-2* mRNAs using a protocol described previously with some modifications (23). mRNAs were isolated from wild-type and mutant strains and resuspended in 30 μL of H<sub>2</sub>O. cDNAs were synthesized from 3 μg of RNA using SuperScript III Reverse Transcriptase (Invitrogen) and 300 ng of oligo(dT)<sub>12</sub>. *TRA-2* oligo 1 and oligo 2 are RT-PCR primers specific to the *tra-2* 3' UTR and were end-labeled with <sup>32</sup>P using polynucleotide kinase (New England Biolabs). A 25-cycle PCR was performed using 20 ng of oligo 1 or oligo 2, oligo(dT)<sub>12</sub> remaining from the cDNA synthesis, and 3 μL of cDNA as templates. The PCR products were analyzed on a 2.5% agarose gel, which was dried and exposed to a PhosphorImaging screen (Perkin-Elmer).

**ACKNOWLEDGMENTS.** We thank Tom Blumenthal, Tom Evans, and Bill Woods for comments on the manuscript. This work is supported by a Burroughs Wellcome Fund Award and by National Institutes of Health R01 Grants GM59083 and GM79097 (to D.X.).

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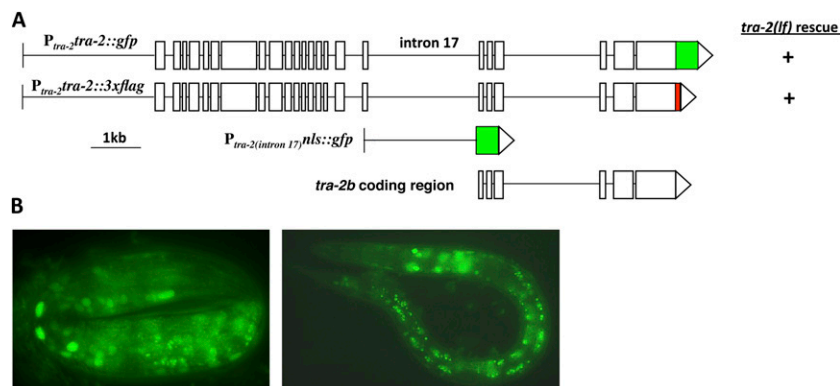
# Supporting Information

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## SI Methods

**Mass Spectroscopy Analysis.** The protein bands of interest excised from silver-stained gels were destained by 1% potassium ferricyanide and 1.6% sodium thiosulfate, subjected to reduction and alkylation by 10 mM DTT/55 mM iodoacetamide in 25 mM  $\text{NH}_4\text{HCO}_3$ , and then in-gel digested with trypsin (20  $\mu\text{g}/\text{mL}$  in 25 mM  $\text{NH}_4\text{HCO}_3$ ) at 37 °C for 16 h. The tryptic peptides were then analyzed by mass spectrometry. For MALDI-TOF mass spectrometric analysis, tryptic peptides were mixed with  $\alpha$ -cyano-4-hydroxycinnamic acid (CHCA) matrix containing 2 fmol internal standards and analyzed on an Ultraflex MALDI-TOF mass spectrometer (Bruker Daltonics). For liquid chromatography-tandem mass spectrometry (LC MS/MS) analysis, each peptide mixture was reconstituted in HPLC buffer A (0.1% formic acid), loaded across a trap column (Zorbax 300SB-C18, 0.3  $\times$  5 mm, Agilent Technologies), and separated on a 10-cm analytical C18 column (inner diameter, 75  $\mu\text{m}$ ; New Objective). The peptides were eluted using a linear gradient of 0–95% HPLC buffer B

(99.9% acetonitrile containing 0.1% formic acid). The LC apparatus was coupled with a 2D linear ion trap mass spectrometer (LTQ-Orbitrap, Thermo Fisher). The resulting spectra from both mass spectrometers were used in searches of the National Center for Biotechnology Information nr database with taxonomy set on *Caenorhabditis elegans* (26,209 sequences) assuming the digestion enzyme trypsin. The MASCOT search engine (<http://www.matrixscience.com>; v.2.2.03 Matrix Science) was used, allowing one missed cleavage site with charge states from 1+ to 3+. MS mass tolerance was set to be 10 ppm for LC-MS/MS (50 ppm for MALDI-TOF MS), and MS/MS tolerance was set to be 0.5 Da for fixed modification for carbamidomethyl cysteines and variable oxidation of methionine residues. Protein identification was performed using Mowse scores ( $P < 0.05$ ) and the MudPIT algorithm of the MASCOT search engine. Peptide identification was performed using the Scaffold 2 search engine (v.2.06.01; <http://www.proteomesoftware.com>).

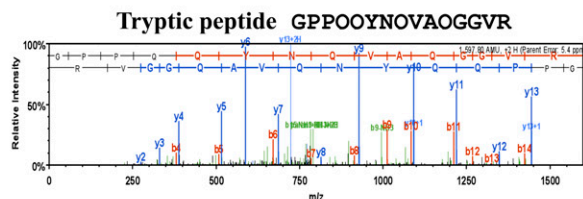


**Fig. S1.** *tra-2* transgenes. (A) Schematic of the *P<sub>tra-2</sub>tra-2::gfp* and *P<sub>tra-2</sub>tra-2::3xflag* translational fusions and the *P<sub>tra-2(intron 17)</sub>nls::gfp* transcriptional reporter. Boxes represent exons, lines indicate introns or promoter, and the triangle represents the 3' untranslated region. The *tra-2b* transcript is also shown. The ability of the translational fusions to rescue the defects of the *tra-2(lf)* mutants is indicated. The *tra-2a* transcript is trans-spliced to the SL2 splice leader, which is consistent with its being a downstream gene in an operon. The *tra-2b* transcript is trans-spliced to the SL1 splice leader, indicating that it is not expressed as a part of the operon (1, 2). The gene upstream of *tra-2* in the operon is *ppp-1*. Green box indicates GFP. Red box indicates the 3xFLAG tag. (B) Images of transgenic embryo and larva carrying the *P<sub>tra-2(intron 17)</sub>nls::gfp* transgene, which directs nucleus-localized GFP expression under the control of the 2071-bp intron 17 of the *tra-2a* isoform.

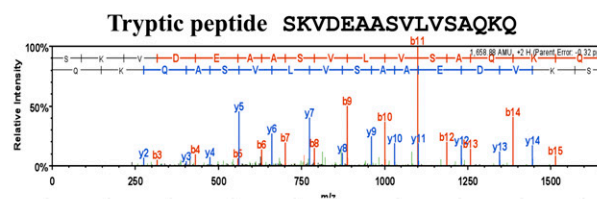
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# A PolyA Binding protein family member (PAB-1) [*Caenorhabditis elegans*] (NCBI Reference Sequence: NP\_001021711.1)

1 MEMNVAAPAA AVAGAAAPQP GQNQTGSSYT MASLYVGDLH PDVNESILFE KFSAGPVL S  
61 IRVCRDNATR LSLGYAYVNF QQPADAERAM DTMNFEALHG KPMRIMWSQR DPAMRRSGAG  
121 NIFIKNLNDKV FVGKFQPRAP RNRELGETAK OFTNVYVKNF GDHYNKETLE KLFKAFGNIT  
181 SCEVMTVEGK SKGFGFVAF NPEEAETAVQ ALHDSTIEGT DLKLHVCRAQ KKSERHAELK  
241 KKHEQHKAE R MQKYQGVNLY VKNLDETVD DGLKKOFESY GNITSAKVMT DENGRSKGFG  
301 FVCFEKPEEA TSAVTEMNSK MCVCSKPLYVA IAORKEDRRA QLASQYMQR L ASMRMHGNVP  
361 GAAMYNPTQP GPGYYVANPM QQQRNFAGGO QMVRPGGRWG MQNQYPVQNG YMMAQGGPGVY  
421 QNRMGRPQNG QGGPRGPPQQ YNOVAQGGVR MQGPPRTQNP GVQQQNVPRP PQQQQQQRP A  
481 PTGPKAPPQP YQAYQQRPG IVIGGQEPLT SAMLAAAAPQ EQKQLLGERI YALIEKLYPG  
541 HKDAGKITGM MLEIDNSEL I MMLQDSELF R SKVDEAASVL VSAQKQ



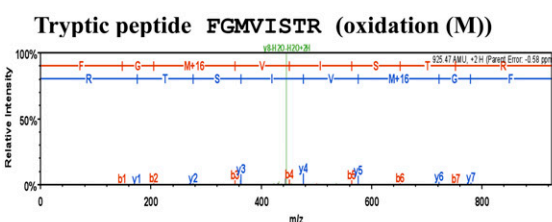
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	58.0				G	1,598.8	799.9	1,581.8		15
2	155.1				P	1,541.8	771.4	1,524.8		14
3	252.1				P	1,444.7	722.9	1,427.7		13
4	380.2		363.2		Q	1,347.7	674.3	1,330.7		12
5	508.3		491.2		Q	1,219.6	610.3	1,202.6		11
6	671.3	336.2	654.3		V	1,091.6	546.3	1,074.5		10
7	785.4	393.2	768.3		N	928.5	464.8	911.5		9
8	913.4	457.2	896.4		Q	814.5	407.7	797.4		8
9	1,012.5	506.7	995.5		V	686.4	343.7	669.4		7
10	1,083.5	542.3	1,066.5		A	587.3	294.2	570.3		6
11	1,211.6	606.3	1,194.6		Q	516.3		499.3		5
12	1,268.6	634.8	1,251.6		G	388.2		371.2		4
13	1,325.6	663.3	1,308.6		G	331.2		314.2		3
14	1,424.7	712.9	1,407.7		V	274.2		257.2		2
15	1,598.8	799.9	1,581.8		R	175.1		158.1		1



B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	88.0				S	1,659.9	830.4	1,642.9	1,641.9	16
2	216.1	108.6	199.1	198.1	K	1,572.9	786.9	1,555.8	1,554.8	15
3	315.2	158.1	298.2	297.2	V	1,444.8	722.9	1,427.7	1,426.8	14
4	430.2	215.6	413.2	412.2	D	1,345.7	673.4	1,328.7	1,327.7	13
5	559.3	280.1	542.2	541.3	E	1,230.7	615.8	1,213.6	1,212.7	12
6	630.3	315.7	613.3	612.3	A	1,101.6	551.3	1,084.6	1,083.6	11
7	701.3	351.2	684.3	683.3	A	1,030.6	515.8	1,013.6	1,012.6	10
8	788.4	394.7	771.4	770.4	S	959.6	480.3	942.5	941.5	9
9	887.4	444.2	870.4	869.4	V	872.5	436.8	855.5	854.5	8
10	1,000.5	500.8	983.5	982.5	L	773.5	387.2	756.4	755.4	7
11	1,099.6	550.3	1,082.6	1,081.6	V	660.4	330.7	643.3	642.4	6
12	1,186.6	593.8	1,169.6	1,168.6	S	561.3		544.3	543.3	5
13	1,257.7	629.3	1,240.6	1,239.7	A	474.3		457.2		4
14	1,385.7	693.4	1,368.7	1,367.7	Q	403.2		386.2		3
15	1,513.8	757.4	1,496.8	1,495.8	K	275.2		258.1		2
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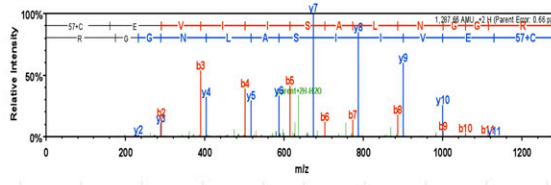
# B *C. elegans* protein R10E4.2a (SUP-26a)[*Caenorhabditis elegans*]. GenBank: CAA90772.1

1 MNASSAPQQQ QQQQQPQQGQ APQQQQQQGP QHQYQQQRPF HGRVQNHMRG SGPFNGNSNGY  
61 GRYTAPRGDQ QHHDSTPLSS TNLVIRGLMP NTNDLLREM CSKYGNIAST KAIMDKATNN  
121 CKGYGFVDFE SPQAAAAVD GLNTEGVQAQ MAKLOQQEQD PTNLIYANLP LDFTEQMLET  
181 ELNKFGMVIS TRILRTPDNQ SRGVGFARM D SKEKCEVIIS ALNGGRFDTM SKEGPALLIK  
241 QADTGRKSKH SMNNPEMLQR MQYPQVYQSY YGYHAAVYQ QHYDVNSLAS QMGGMHVGGG  
301 NPQANGGDM VIGAHMYGQNA GGQGGQGVGQ GGQGGNQFY NPNNGRNKKY FQPMNPQ



B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	148.1				F+16	926.5	463.7	909.5	908.5	8
2	205.1				G	779.4	390.2	762.4	761.4	7
3	352.1				M	722.4	361.7	705.4	704.4	6
4	451.2				V	575.4		558.3	557.3	5
5	564.3				I	476.3		459.3	458.3	4
6	651.3	326.2		633.3	S	363.2		346.2	345.2	3
7	752.4	376.7		734.4	T	276.2		259.1	258.2	2
8	926.5	463.7	909.5	908.5	R	175.1		158.1		1

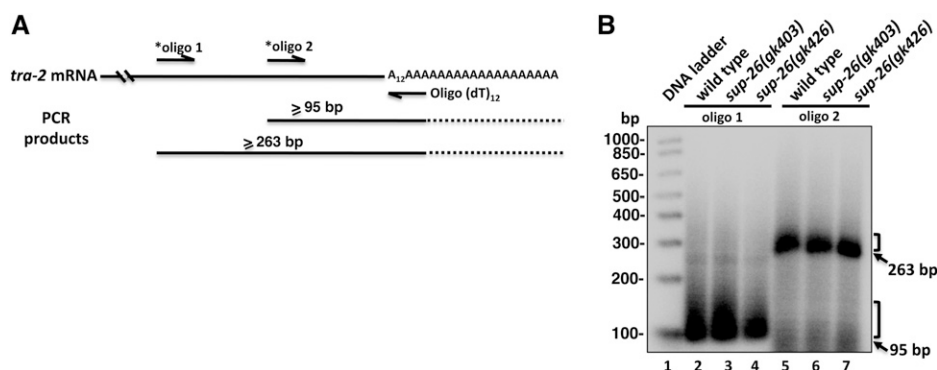
# Tryptic peptide CEVIISALNGGR (carbamidomethyl (C))



B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	161.0				C+57	1,288.7	644.8	1,271.6	1,270.7	12
2	290.1				E	1,128.6	564.8	1,111.6	1,110.6	11
3	389.1				V	999.6	500.3	982.6	981.6	10
4	502.2				I	900.5	450.8	883.5	882.5	9
5	615.3				I	787.4	394.2	770.4	769.4	8
6	702.3	351.7		684.3	S	674.4	337.7	657.3	656.3	7
7	773.4	387.2		755.4	A	587.3		570.3		6
8	886.5	443.7		868.5	L	516.3		499.3		5
9	1,000.5	500.8	983.5	982.5	N	403.2		386.2		4
10	1,057.5	529.3	1,040.5	1,039.5	G	289.2		272.1		3
11	1,114.6	557.8	1,097.5	1,096.5	G	232.1		215.1		2
12	1,288.7	644.8	1,271.6	1,270.7	R	175.1		158.1		1

**Fig. S2.** Representative MS/MS spectra from LTQ Orbitrap used to confirm the identification of poly(A)-binding protein PAB-1 and SUP-26 in the SUP-26::GFP-containing immunoprecipitated complexes. Tryptic peptides of protein bands 1 and 2 in SDS/PAGE (Fig. 5A) were analyzed by LC-MS/MS using LTQ Orbitrap. The amino acid sequences of peptides identified by MS/MS analysis and matched to the amino acid sequences of PAB-1 (A) and SUP-26 (B) are underlined. The representative MS/MS spectra of two selected peptides (indicated in red) for each protein are shown below the amino acid sequences. The assignments of the fragmented ions observed to specific amino acid residues were performed using the Scaffold 2 search engine, and the search results are shown below the MS/MS spectra.





**Table S3. MALDI-TOF mass spectroscopic analysis of protein bands detected in the SUP-26::GFP-containing immunoprecipitated complexes**

Band no./ protein name	M.W. (kDa)	Mascot score	Sequence coverage rate (%)	Accession no.	Peptide: start– end	Mass expected	Mass calculated	$\Delta$ Mass (ppm)	Peptide sequence matched
1/PAB-1	71.945	59	21%	gil71993203	52–62	1116.6366	1116.6291	7	K.FSAAGPVLISIR.V
					71–88	2040.9812	2040.9905	–5	R.LSLGYAYVNFQPPADAER.A
					89–104	1863.8457	1863.8430	1	R.AMDTMNFEALHGKPMR.I Oxidation (M)
					180–189	1029.5899	1029.6005	–10	K.VNGMLLAGKK.V
					211–226	1972.8880	1972.9432	–28	K.QFTNVYVKNFQDHYNK.E
					381–394	1634.8166	1634.8636	–29	K.MVCSKPLYVAIAQR.K
					399–409	1350.6494	1350.6826	–25	R.RAQLASQYMQR.L
					400–409	1194.5858	1194.5815	4	R.AQLASQYMQR.L
					445–458	1473.7263	1473.7259	0	R.NFAGGQQMVRPGGR.W
					484–495	1324.6403	1324.6418	–1	R.MGRPQNQQGGPR.G
2/TAG-310 (SUP-26)	39.455	76	25%	gil17554534	496–510	1597.7986	1597.7961	2	R.GPPQQYNQVAQGGVR.M
					50–62	1268.5529	1268.5534	0	R.GSGPFGNSNGYGR.Y
					68–86	2168.0329	2168.0247	4	R.GDQQHHDSTPLSSTNLYIR.G
					87–98	1357.6623	1357.6660	–3	R.GLMPNTNDDLRL.E
					87–98	1373.6593	1373.6609	–1	R.GLMPNTNDDLRL.E oxidation (M)
					87–103	1992.8794	1992.9067	–14	R.GLMPNTNDDLREMCCK.Y
					185–192	909.4837	909.4742	10	K.FGMVISTR.I
					185–192	925.4767	925.4691	8	K.FGMVISTR.I oxidation (M)
					213–226	1544.7862	1544.7981	–8	K.EKCEVIISALNGGR.F
					215–226	1287.6580	1287.6605	–2	K.CEVIISALNGGR.F
					233–240	839.5134	839.5116	2	K.EGPALLIK.Q
					248–260	1570.7263	1570.7344	–5	K.SKHSMMNPEMLQR.M
					250–260	1355.6149	1355.6074	6	K.HSMNPEMLQR.M
					250–260	1371.6174	1371.6023	11	K.HSMNPEMLQR.M oxidation (M)

Tryptic peptide mass fingerprints of protein bands 1 and 2 in the SDS/PAGE (Fig. 5A) were analyzed by MALDI-TOF mass spectrometry. When searched in the National Center for Biotechnology Information nr database with taxonomy set on *C. elegans*, these observed peptide masses matched to 11 peptides of PAB-1 and to 13 peptides of TAG-310 (SUP-26), with a 21% and a 25% sequence coverage rate, respectively. The corresponding amino acid residue numbers (start to end) of matched peptides in PAB-1 and SUP-26 are denoted. The Mascot score used for protein identification is defined as  $-10 \times \log(P)$ , where  $P$  is the probability that the observed match is a random event. Scores greater than 57 are considered to be significant ( $P < 0.05$ ).  $\Delta$ Mass (in ppm) indicates the difference between expected (expt) mass and calculated (calc) mass, which is defined as  $[\text{mass}(\text{expt}) - \text{mass}(\text{calc})]/\text{mass}(\text{calc}) \times 1,000,000$ . M.W., molecular weight.

**Table S4. Oligonucleotides used in this study**

Oligonucleotide	Sequence
SUP-26proS	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGCTAGCTCGCAGAGCCTAAAAACATTTC 3'
SUP-26NS	5' GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGAACGCATCTTCGGCTCCACAACAAC 3'
SUP-26CAS	5' GGGGACCACTTTGTACAAGAAAGCTGGGTGTTGTGGATTTCATCGGCTGGAAATA 3'
TRA-2proS	5' CGGGATCCCATGGTGAGGCCT 3'
TRA-2proAS	5' AAATCCCGGGTTTATCTGAAATTGTTATTTGAACG 3'
TRA-2 3'S	5' ATGAATTCAATGTCTGTTTCCTTTTCAG 3'
TRA-2 3'AS	5' ATATACTAGTTAACTATTACGTTTATTAAC 3'
TRA-2 3'T7 S	5' TAATACGACTCACTATAGGGAATGTCTGTTTCCTTTTCAGAATTTGG 3'
TRA-2 delta TGE S	5' TGTACAATTTCCATTTCATATCGTCCACTCGAC 3'
TRA-2 delta TGE AS	5' GTCGAGTGGACGATATGAAATGGAAATTGTACA 3'
oligo(dT) <sub>12</sub>	5' GCGAGCTCCGCGCGCGCTTTTTTTTTTTT 3'
TRA-2 oligo 1	5' AATGTCTGTTTCCTTTTCAGAATTTGG 3'
TRA-2 oligo 2	5' ATCGTCCACTCGACCTCAACTGTAAT 3'